This is the final pre-publication version of:
Cashman M.J., Pilotto F., Harvey G.L., Wharton G. and Pusch M.T. (2016) Combined stable isotope and fatty acid analyses demonstrate that large wood increases the autochthonous trophic base of a macroinvertebrate assemblage. *Freshwater Biology*. DOI: 10.1111/fwb.12727

# Combined stable isotope and fatty acid analyses demonstrate that large wood increases the autochthonous trophic base of a macroinvertebrate assemblage

*Matthew J. Cashman*[*,1,2,3], *Francesca Pilotto*[*,1,2,3], *Gemma L. Harvey*[3], *Geraldene Wharton*[3], *Martin T. Pusch*[1]

[1]*Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany*

[2]*Institute of Biology, Freie Universität Berlin, Berlin, Germany*

[3]*School of Geography, Queen Mary University of London, London, U.K.*

* Co-first authorship: MJC and FP equally contributed to the study

Email address: matthewjcashman@gmail.com

*Running Header:* Large wood increases the autochthonous trophic base

*Keywords:* macroinvertebrates, trophic resources, lake-river coupling, sand-bed river, food webs

**Summary**

1. Large wood (LW), defined as pieces of wood greater than 10 cm in diameter and 1 m long, is well known to alter river hydromorphology and the availability of potential food resources for consumers. However, there has been a lack of studies investigating whether these can cause shifts in the trophic base, which may explain alterations to the total abundance and taxonomic structure of the macroinvertebrate assemblage.
2. We aimed to determine how the presence of large wood altered the trophic base of the macroinvertebrate consumer assemblage in a lowland river, and to provide a methodological comparison of two assimilation-based food web methods: stable isotope analysis (SIA) and fatty acid biomarker profiles (FA). To do so, we quantified the contribution of trophic resources to the diets of macroinvertebrates colonizing the surface of LW, present in this study as single logs, and surrounding bed sediments with those from bed sediments of a nearby control site with minimal amounts of LW.
3. SIA showed that the macroinvertebrate food web, even for non-filter feeding taxa, was mostly sustained by seston exported from a lake 1 km upstream, highlighting a high degree of lake-river coupling. The presence of wood altered the trophic base from being predominantly seston-supported to one with increased support from epixylic autochthonous production (*i.e.* periphyton and bryophytes on wood). Terrestrial matter (*i.e.* leaves and grass) and organic sediments were a relatively unimportant fraction of the trophic base (<10%) in all locations.
4. FA did not directly track the influence of seston, but instead differentiated between overall allochthonous (terrestrial) and autochthonous (aquatic) components of the trophic base. In particular, FA analysis demonstrated the higher nutritional value of autochthonous primary producers, and provided supporting evidence that most consumers, even seston-feeders, were primarily supported by autochthonous resources and not by allochthonous matter. FA indicated shifts in some taxa-specific diets not detected by stable isotopes alone.
5. Our study demonstrated that the combined use of stable isotopes and fatty acids provides new insights into determining the trophic base of a complex food web with trophic

resources of both terrestrial/aquatic and lacustrine/riverine origins. In addition, directly comparing results from both stable isotope and fatty acid analyses provided additional information on selective feeding by seston-feeding taxa on autochthonous and allochthonous fractions of the seston.

6. The presence of large wood in the river channel decreased lake-river coupling by providing alternative basal resources, primarily through increasing high quality autochthonous production on wood and by providing a superior substratum for net-spinning caddisflies to feed on a fraction of the seston richer in essential fatty acids. River management strategies that incorporate instream large wood therefore have the potential to alter energy flows and enhance ecosystem productivity by increasing the quantity and quality of available basal food resources.

## Introduction

Large wood (LW), usually defined as wood pieces in the river channel larger than 10 cm in diameter and 1 m in length (Gippel *et al.*, 1996) and often referring to whole logs fallen into or across a stream channel (Gurnell *et al.*, 2002; Gurnell *et al.*, 2000; Reeves, Burnett & McGarry, 2003), constitutes a fundamental component in the health and integrity of river ecosystems. LW functions as an element of structural complexity in the channel, increasing the heterogeneity of physical habitat conditions (Ehrman & Lamberti, 1992; Gregory, Gurnell & Petts, 1995; Montgomery *et al.*, 1995; Gurnell & Linstead, 1998). LW has been shown to increase macroinvertebrate assemblage diversity by providing a stable and hard substratum for colonisation (Hoffmann & Hering, 2000; Benke & Wallace, 2003; Schröder *et al.*, 2013) and by providing diverse habitats in nearby river-bed sediments (Pilotto *et al.*, 2014). Large wood may also influence the local abundance and composition of consumers by affecting the availability and quality of heterogeneous food resources. LW can serve directly as a food source for xylophilic macroinvertebrate species, but the proportion of these taxa is relatively low (Anderson *et al.*, 1978; Anderson, Steedman & Dudley, 1984; Anderson, 1989; Hoffman & Hering, 2000). LW may also alter the availability of both allochthonous (terrestrial) and autochthonous (aquatic) food resources present in the channel. Much research has focused on the ability of LW to increase organic matter retention by trapping fine sediment, leaves, twigs and other transported matter (Bilby & Likens, 1980; Bilby, 1981). In addition, the erosion and decay of the wood surface can contribute to increased organic matter within the reach (Ward & Aumen, 1986). LW also directly increases the total surface area of hard substratum for colonisation by biofilm (Hax & Golladay, 1993; Wondzell & Bisson, 2003), and the rough surface texture of wood can result in increased algal diversity and unique species assemblages, particularly for taxa sensitive to shear stress (Sabater, Gregory & Sedell, 1998). This may be particularly important in sand-bed rivers, as stable and hard substrata besides LW may be otherwise limited in the channel. However, few studies have directly investigated whether these changes to food availability shift the trophic base of the macroinvertebrate assemblage.

Stream ecosystems with dense riparian shading have been hypothesised to be primarily supported by allochthonous production due to light-limitation of in-stream production and high inputs of terrestrial matter (Vannote *et al.*, 1980; Smock, Metzler & Gladden, 1989). However, recent work on the trophic base of macroinvertebrate assemblages have suggested that terrestrial matter may contribute a relatively minor fraction of the diet, with macroinvertebrates largely dependent on autochthonous matter, even for species generally considered shredders (Torres-Ruiz, Wehr & Perrone, 2007; Lau, Leung & Dudgeon, 2009). Allochthonous carbon is mostly recalcitrant, while autochthonous production, although less plentiful, is more labile and contains higher concentrations of nitrogen, phosphorus and specifically highly unsaturated fatty acids (HUFAs)(Brett & Müller-Navarra, 1997; Thorp & Delong, 2002; Torres-Ruiz, Wehr & Perrone, 2007). A high-quality food base rich in HUFAs is suggested to enhance energy transfer from basal resources to consumers, whereas a lack of these important components may lead to trophic decoupling, whereby increased primary production does not result in increased production at higher trophic levels (Brett & Müller-Navarra, 1997; Müller-Navarra *et al.*, 2000; Gladyshev *et al.*, 2011; Perhar, Arhonditsis & Brett, 2013; Taipale *et al.*, 2014). While the increased residence time of organic matter trapped by LW may increase microbial enrichment and the quality of allochthonous matter for the food web (Smock, Metzler & Gladden, 1989; Fry & Fuller, 1991), even limited increases in autochthonous production associated with LW may have large proportional effects on the trophic base of the macroinvertebrate assemblage.

Stable isotope analysis (SIA) of carbon and nitrogen retention and fractionation has become a standard method in evaluating aquatic food webs, with Bayesian mixing models providing quantitative estimates of mixed diet composition (Moore & Semmens, 2008; Parnell *et al.*, 2008; Ward *et al.*, 2011; Parnell *et al.*, 2013). Combining SIA results with fatty acid biomarker profiles can facilitate the interpretation of food web structure, particularly in situations where some of the basal resources have overlapping isotope signatures or in cases of mixed trophy (El-Sabaawi *et al.*, 2009; Allan *et al.*, 2010; Galloway *et al.*, 2012). Rather than the two- or three-source signals (C,N,S) used in traditional stable isotope analysis, FA profiles use more than a dozen fatty acids that are synthesized in biologically relevant amounts by particular phylogenetic lineages (*e.g.*

bacteria, diatoms, green plants). These profiles are then retained in consumers and can be used to trace trophic relationships through the aquatic environment, even in cases of omnivory (Gladyshev, Arts & Sushchik, 2009).

We aimed to quantify the effect of LW on the trophic base of a river ecosystem by combining analyses of stable isotope and fatty acid biomarker profiles of the macroinvertebrates and their potential trophic resources on and around LW compared to areas of the channel without wood. We hypothesised that the presence of LW would support the growth of epixylic biofilms that would result in increased autochthonous support in the average diet of the benthic invertebrate assemblage around wood.

## Methods

*Study area*

Field work was carried out in April 2012 in the Płociczna River, a lowland, minimally-disturbed sand-bed river in the Drawienski National Park in Western Poland (Fig. 1). The Drawienski National Park is in the southern part of the Pomeranian Lake District, with a geology of early-glacial outwash plains and land cover of mixed coniferous plantation and hardwoods. The Płociczna runs for 51 km until its confluence with the Drawa River, and the dominant riparian vegetation consists of broad-leaved trees, mainly alder (*Alnus* sp.). We studied two forested reaches (bankfull width: 12-15 m; near-bankfull discharge: 1.4-1.5 $m^3 s^{-1}$), with varying levels of LW (Fig. S1) that were located downstream of Lake Sitno, a 67 $ha^{-1}$ eutrophic throughflow lake. The upstream reach (ca. 700 m from the lake outflow) had little in in-channel LW (nine small wood structures in 100 m, with a total volume of 22.9 $m^3 ha^{-1}$ of channel area, primarily bark and twigs; hereafter “wood-poor site”), while a downstream reach (ca. 1000 m from the lake outflow) had abundant in-channel LW (25 wood structures in 100 m, with a total volume of 94.4 $m^3 ha^{-1}$ of channel area; hereafter “wood-rich site”). Overall, LW pieces consisted of whole logs fallen into the stream channel, covered in bark, both with and without branches, and uniformly aligned perpendicular to channel flow. Due to the low gradient and limited stream power of the studied reach, there was no evidence of large wood having been transported or re-orientated by flow.

*Food resources*

Basal food resources were collected in three replicate samples from each site and included wood, grass, leaf litter, sediments, bryophytes, filamentous algae, periphyton collected on wood, periphyton collected on mussel shells, and transported organic matter ("TOM"). Leaf litter and grass were collected from the riparian zone. The top 5-cm of sediment (sand and organic matter deposits) were collected with a Perspex sediment core. Bryophytes were collected from both wood and on the river banks. The filamentous green alga, *Cladophora* sp., which was only found in the wood-rich reach, was collected from submerged pieces of wood and cleaned of organic matter and epiphytes under a 10x dissecting microscope in the laboratory. Periphyton on wood was also collected from submerged pieces of large wood, which was removed to the river bank and sampled using a toothbrush. In the wood-poor site, only small pieces of woody material (*i.e.* bark, small branches) were present, which were also sampled for periphyton. After periphyton was removed from the wood surface, wood fragments were broken and removed with a razor blade and screwdriver for the cleaned wood sample. Periphyton on mussels was collected in the wood-poor reach with a toothbrush, as mussels were the only other hard substratum available in the channel. All periphyton slurries were collected in vials and put on ice until return to the laboratory. TOM was collected mid-river with a 125 µm phytoplankton net over 30 minutes at three equidistant points (upstream, mid-point and downstream) along 100 m of each reach. The isotopic signature of total seston was further inferred from the isotopic signature of bulk unionid mussel tissue ("unio-derived seston": *Unio tumidus* and *Unio pictorum*) collected from the two study sites; unionid mussels are often used as a time-integrated seston signature (Cabana & Rasmussen, 1996; Atkinson *et al.*, 2014), since unionids are long-lived sestonic filter feeders and thus their tissue is less sensitive to seasonal fluctuations in the values of carbon and nitrogen stable isotope ratios. All samples were brought to the laboratory, where they were washed under filtered water, cleaned under a microscope (20x) in order to remove animals and organic material, and prepared for stable isotope and fatty acid analyses. Periphyton and TOM slurries were filtered onto pre-ashed 25-mm Whatman® GF/F filters (Sigma Aldritch Chemie Gmbh, Munich, Germany) and leaves and grass were ground into a fine powder with a ball tissue grinder.

111 *Sampling for macroinvertebrates*

112 Macroinvertebrates were collected in the wood-rich site from the LW surface (WW samples) and
113 bed-sediment within 20 cm of LW (WS samples), and in the wood-poor site from the bed-sediment
114 away from any wood (NW samples).

115 For analysis of the macroinvertebrate taxonomic composition we selected six replicate pieces of
116 LW within the wood-rich-site. We collected one sample from the surface of each LW by brushing
117 an area of 0.26 $m^2$ into a hand net. We collected benthic samples from the sediment around each
118 selected LW at three sampling points: one upstream, one downstream, and one lateral to the LW.
119 We additionally collected six replicate benthic samples in the wood-poor site. Each benthic sample
120 consisted of the pooled material from five Surber samplers (frame size: 23x23 cm, mesh size: 500
121 µm; total sampled area of each sample: 0.26 $m^2$). Samples were preserved in 70% ethanol, and in
122 the laboratory animals were identified to species or genus. Taxa abundances from the three
123 sampling points on the river-bed sediments surrounding the same LW (upstream, downstream and
124 lateral) were averaged in order to obtain a composite sample for the area surrounding each of the
125 six replicate LW.

126 We collected three additional replicate invertebrate samples from the LW surface (WW), three from
127 the sediment surrounding LW (WS), and three from bed-sediment in the wood-poor site (NW) for
128 isotopes and fatty acid analyses. The samples were sorted in the field and transported in filtered
129 river water to the laboratory where they were identified under a 10x microscope and left for 24 h for
130 gut clearance. When the number of animals sufficed, half of the sample of each taxon was
131 processed for stable isotope analysis and half for fatty acid analysis (Table 1).

132 *Sample processing for stable isotope analysis*

133 Trophic resources and single (large animals: *e.g.* Odonata) or pooled (small animals: *e.g.*
134 Chironomidae) macroinvertebrate individuals belonging to the same taxon were dried separately at
135 60 °C for 48 h, weighed and ground to a fine powder. Subsamples of ~1mg for animals and from 1
136 to 30 mg for food resources were placed in tin capsules and sent for analysis at the UC Davis

Stable Isotope Facility, where they were analysed using mass spectrophotometry. Stable isotope data are expressed in δ notation (‰) as the relative difference between ratios of samples and international standards (Vienna PeeDee Belemnite and air for carbon and nitrogen, respectively).

*Sample processing for fatty acid profiles*

All samples for fatty acid analyses were stored at -80°C under $N_2$ until extraction following a method adapted from Torre-Ruiz *et al.* (2007) and originally modified from Parrish (1999). Samples were extracted in 2 washes of chloroform:methanol (2:1 v/v), sonicated on ice, and the chloroform phase was separated for methylation into fatty acid methyl esters with $BF_3$ (10 -14% w/v in methanol) at 80°C. Fatty acid methyl esters were suspended in hexane and measured on an Agilent 6890 gas chromatograph with an Agilent 5973-N mass selective detector that was fitted with a CP Sil 88 for FAME fused-silica capillary column (100m x 250 µm x 39 µm) set in splitless mode. Carrier gas (He) flow rate was constant at 0.2 mL $min^{-1}$. Inlet temperature was 300°C, with initial temperature 70°C with an increase of 720°C $min^{-1}$, and detector temperature was set at 280°C. The temperature program started at 80°C for 1 min, increased at a rate of 4°C $min^{-1}$ until reaching a temperature of 220°C. This was maintained for 4 min, heated at 4°C $min^{-1}$ until 240°C, where it was maintained for a final 15 min. The total temperature program lasted for 60 minutes. Fatty acid methyl esters were identified by retention times and mass spectra in full scan mode previously calibrated with standards: 37-Component FAME Mix (47885-4), PUFA No1; Marine Source (47033) and PUFA No3; Menhaden Oil (47085-4; all Supelco, Germany).

*Data analysis*

Community composition of the macroinvertebrate assemblages colonising the three substrata (NW, WS and WW) was compared by non-metric multidimensional scaling (nMDS) and analysis of similarities (ANOSIM) using the package vegan in R (Oksanen *et al.*, 2013). These analyses were run on log(x+1)-transformed macroinvertebrate data with Bray-Curtis distance among samples. We also computed Shannon-Wiener diversity indices and the rarefied taxonomic richness using the functions also implemented in the R package vegan. The values of those metrics and the total

abundances were compared among the three groups of samples (NW, WS and WW) through analysis of variances (ANOVA).

We estimated the relative importance of the trophic sources to the diet of the studied macroinvertebrate taxa using mixing models implemented in the SIAR package in R (Parnell *et al.*, 2008; Parnell *et al.*, 2010). Such models are based on a Bayesian approach and estimate the probability distributions to a consumer diet starting from the $\delta^{13}C$ and $\delta^{15}N$ signature of each consumer, that of each source (mean ± standard deviation) and the trophic enrichment factor (TEF). We used the TEF values reported by Post (2002), *i.e.* 0.4 ± 1.3 ‰ for $\delta^{13}C$ and 3.4 ± 1.0 ‰ for $\delta^{15}N$. For predator taxa, we doubled the TEF values. Since *Hydropsyche* sp. can show both primary consumer and predatory behaviour, we included in the model both TEF and doubled TEF values, and the results were combined *a-posteriori*. We ran the models for each taxon including all trophic resources that were present at the site. If two sources are located in the same isotopic space, it may be impossible for the model to determine the differences in their contributions (Ward *et al.*, 2011; Parnell *et al.*, 2013). To account for that, the models were checked for correlations among resources (by using the function “siarmatrixplot” of the R package SIAR) and the resources that showed strong (>60) negative correlations in at least one model were *a-posteriori* combined (Ward *et al.*, 2011; Parnell *et al.*, 2013). Thus seston inferred from the isotopic signature of unionid mussels was combined with filamentous algae because they were negatively correlated in several models, thus forming the group “unio-derived seston and filamentous algae”. Epixylic periphyton and bryophytes were also combined because they were negatively correlated in several models (epixylic autochthonous material). Grass and leaves were considered separately in the SIAR models, but the results were then *a-posteriori* aggregated as a collective “terrestrial source” since we were interested in the relative contribution of the allochthonous riparian subsidies. Wood was considered separate from the "terrestrial source" group as it requires a specialized feeding behaviour.

We up-scaled the stable isotope results obtained for single taxa to the community level by weighting the diet composition of the single taxa (output of the SIA mixing models) by their mean

biomass within the assemblage. The mean biomass of each taxon was computed from the average of individual dry weight of the samples that were dried for isotope analysis, multiplied by the mean abundance of that taxon.

We included all fatty acids in the analysis greater than 1% of all quantified fatty acids. The fatty acid profiles of both basal resources and consumers were ordinated using nMDS using percent composition of all quantified fatty acids. Differences among the overall profiles were compared using ANOSIM, and similarity percentage analysis (SIMPER) was used to determine the specific fatty acids responsible for the difference between profiles. Fatty acid profiles for wood and leaves, being *Alnus* sp., were grouped for the ordination. The nMDS, ANOSIM, and SIMPER were conducted in the R package vegan (Oksanen *et al.*, 2013).

Fatty acids were subdivided into the four major fatty acid classifications: saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), 18 C polyunsaturated fatty acids (PUFA), and ≥20 C highly-unsaturated fatty acids (HUFA), in addition to bacterial fatty acids (BrFA), the sum of quantified bacterial fatty acids in this study (*i.e.* 15:0 and 17:0), as has been done in previous studies (Rajendran, Suwa & Urushigawa, 1993; Alfaro *et al.*, 2006). The ratio between the sum of all omega-3 and omega-6 fatty acids was also calculated as an indicator of the influence of allochthonous or autochthonous matter in the diet (Torres-Ruiz, Wehr & Perrone, 2007; Taipale, Kainz & Brett, 2015). For basal resources, total fatty acid content was listed per unit dry weight of the basal resource, to indicate FA content ingested by consumers per unit weight of food, and as a percentage to examine indicative biomarkers. Consumer fatty acid content was also examined as a percentage of all fatty acids to determine diet biomarkers. Differences in fatty acid classes and trophic biomarkers were compared across basal resources and across mesohabitat locations for individual consumers, using ANOVA in the R package car (Fox *et al.*, 2012). Post-hoc tests were examined with an F-test with holm p-adjustment using the testInteractions function in R package phia (Rosario-Martinez, 2013). Significance level was set for all tests at $\alpha < 0.05$.

**Results**

A total of 32 taxa was collected from the three sampled substrata (Table 1), each of which was colonised by a different macroinvertebrate assemblage (ANOSIM: R= 0.92, p= 0.001; Fig. 2). Chironomidae was the dominant taxonomic group in all three datasets, representing on average 72% of the abundance in both wood-poor (NW) and wood-rich sediments (WS), and 80% of the total abundance on the wood surface (WW). The second most abundant group was *Caenis* sp. in the wood-poor and wood-rich sediments (16% and 7% respectively of total abundance), and Oligochaeta on the wood surface (14% of total abundance). The highest total invertebrate abundances were recorded on the wood surface (mean 12.8 ± 6.9 $x10^3$ individuals $m^{-2}$; mean ± s.d.), followed by wood-rich sediments (7.4 ± 2.8 $x10^3$) and least in wood-poor sediments (6.4 ± 4.1 $x10^3$), although this was not significantly different (ANOVA, F= 2.97, df= 2, p=0.08). The highest rarefied taxa richness (ANOVA, F= 43.68, df= 2, p<0.01) was recorded in sediments in the wood-rich site (25.5±4.0 $m^{-2}$), followed by the wood-poor site (16.3±1.9) and then the LW surface (11.0 ± 1.7). The highest values of Shannon-Wiener diversity index (ANOVA, F= 12.36, df= 2, p<0.01) were recorded on the sediment around LW in the wood-rich site (1.53 ± 0.18 $m^{-2}$) followed by the wood-poor site (1.17 ± 0.18) and the LW surface (1.11 ± 0.06).

*Stable isotopes: diet composition of the studied taxa*

All consumers were extremely $^{13}C$-depleted, with average $\delta^{13}C$ values of -35.6 ± 0.2 ‰, while most food resources had appreciably higher $\delta^{13}C$ ranging from -31.5 to -21.7‰ (Fig. 3). Periphyton on mussels had the highest $\delta^{13}C$ of all food resources, ranging from -21.7 to -27.9‰, while filamentous algae (-39.9 to -38.5‰) and unio-derived seston (-37.0 to -35.7‰) had the lowest $\delta^{13}C$. The stable isotope mixing model indicated that unio-derived seston/filamentous algae were the dominant basal resources for all taxa on the three substrata (ranging from 41% to 75% of macroinvertebrate diets), with the exception of Oligochaeta that showed similar contributions to the diet from periphyton/bryophytes (See Fig. S2). The contribution of periphyton/bryophytes to the diet of specific macroinvertebrate taxa ranged from 8%-49% and that of grass/leaves from 6%-26%, while the other food resources represented only minor fractions. The diets of specific

macroinvertebrate taxa did not significantly differ across the three different substrata (as shown by the overlap of 95% credible intervals).

*Stable isotopes: trophic bases of the macroinvertebrate assemblages*

The assemblage level analyses indicated that, although unio-derived seston/filamentous algae were the most important resources in all three substrates, the contribution of the different trophic resources to macroinvertebrate biomass greatly differed among the three substrates (Fig. 4). The total biomass supported by unio-derived seston/filamentous algae increased with increasing proximity to wood (NW<WS<WW).

Epixylic material (periphyton and bryophytes) increased in importance in the diet with increasing proximity to wood, supporting a 1.4-fold increased macroinvertebrate biomass in wood-rich sediment and a 4.3-fold increase on the wood-surface compared to NW. Those differences were statistically significant as shown by the lack of overlap of 95% credible intervals (Fig. 4). Grass and leaves also supported higher biomass on the LW surface (13.75±1.62 mg $m^{-2}$) and on river-bed sediments around the LW in the wood-rich site (14.42±1.37 mg $m^{-2}$) than in the wood-poor site (7.54±0.54 mg $m^{-2}$; Fig. 4), although such difference was not significant (overlap of credible intervals).

*Fatty Acids*

The major fatty acid constituents, 14:0, 16:0, 18:0, 18:1ω9c (oleic acid, OA), and 20:5ω3 (eicosapentaenoic acid, EPA) accounted for 60% of the total fatty acids in the study. However, the proportion of these FAs, and other important FA biomarkers, varied considerably across the basal resources and taxa examined.

Fatty acid profiles of the basal resources were not significantly different between wood-rich and wood-poor reaches. Autochthonous sources, with the exception of filamentous algae, contained the most total fatty acids by weight (Table 2). Periphyton on mussels had the most fatty acids available for consumers (51.93±4.97 mg $g^{-1}$ dry weight); in contrast, organic sediments had fatty acid concentrations nearly an order of magnitude smaller (5.68±2.43 mg $g^{-1}$; Table 2). Saturated

fatty acids (SAFAs) were the most abundant fatty acid class across all basal resources (48 – 68%), and highly unsaturated fatty acids (HUFAs) were the least abundant fatty acid class on average, although this was highly variable by source (periphyton on mussels: 19.1±0.9% – Grass: 4.6±0.6%;Table 2).

A non-metric multidimensional scaling (nMDS) ordination of the fatty acid profile roughly separated the available basal resources into three groups: 1) periphyton on mussels, 2) periphyton on wood and bryophytes, and 3) filamentous algae, wood/leaves, grass, and sediment (Fig. 5).

Periphyton on mussels was characterized by the greatest ω3:ω6 ratio (3.5) and high levels of HUFA (19.1±0.9%), particularly of eicosapentaenoic acid (EPA: 20:5ω3; 12.4±0.8%) and docosahexaenoic acid (DHA: 22:6ω3; 2.3±0.1%; Table 2). Periphyton on wood and bryophytes contained lower ω3:ω6 ratios (2.5±0.3 and 1.9±0.3) than periphyton on mussels, but these were still significantly greater than in allochthonous sources (Table 2), and periphyton on wood contained the second-highest levels of EPA quantified in the study (8.0±0.8%) and bryophytes contained the highest levels of both arachidonic acid (ARA: 20:4ω6; 2.7±0.3%) and DHA (2.5±0.7%; Table 2).

Terrestrial matter and sediments contained substantially less HUFA than autochthonous sources (Table 2). The ratio of omega-3:omega-6 fatty acids (ω3:ω6) was lowest in allochthonous sources, near or below 1, and sediment was slightly greater than 1 (Table 2). Filamentous algae, which were collected only in wood habitats, had a similar fatty acid profile to terrestrial material (grasses, wood, and leaves), although one sample contained a profile similar to periphyton on wood. Transported organic matter (TOM) was also highly variable between samples, with several samples similar to sediments and two with a greater autochthonous signal. TOM also exhibited the greatest levels of bacterial fatty acids seen in the study (5.3±0.6%) and the ω3:ω6 ratio was 1.35±0.24, indicating a composition predominately consisting of allochthonous sources.

Most macroinvertebrate taxa had fatty acid profiles similar to the range of available food resources, with most consumers having similar signatures to bryophytes/periphyton on wood and periphyton on mussels (Fig. 5). Among Trichoptera, all had high autochthonous signatures and were ordinated

near periphyton on mussels. The net-spinning caddisfly *Hydropsyche pellucidula* was present in all three locations, while the trumpet-net caddisfly from the group Polycentropodidae was found exclusively in NW. *H. pellucidula* had substantially different fatty acid profiles in wood-rich and wood-poor locations, suggesting different diets. Both WS and WW samples grouped closely with periphyton on mussels, contained high levels of HUFA and ω-3 fatty acids, and were significantly different from NW locations (Fig. 5; ANOSIM: R = 0.682, P = 0.002). Compared to NW, *H. pellucidula* in wood locations had increased 12:0, 2-fold greater EPA, 16:1, 18:3ω3, and 2-fold greater DHA, but decreased 16:0, 18:0, 18:1ω9, and 14:1 (SIMPER: 80%, descending order of importance; Table S1). However, the ω3:ω6 of *H. pellucidula* in NW, even though lower than in both wood habitats, was still extremely high (2.9±0.1), indicating a diet dominated by autochthonous sources (Table S1).

Diptera primarily comprised Chironomidae, which were significantly different among all three sampling locations, although this separation was not along any clear resource gradient (Fig. 5; ANOSIM: R = 0.589, P = 0.006). WW Chironomidae were located centrally in the plot near periphyton on wood, while NW Chironomidae were located to the lower right, and WS Chironomidae to the upper left, overlapping with the bryophyte signal. In comparison to NW, WW had decreased 16:1ω9, 14:0, 18:2ω6c and 18:3ω3, but increased concentrations of 16:0, 18:0, 14:1, EPA, and 12:0 (SIMPER NW-WW: 82%). WS Chironomidae in comparison to NW Chironomidae contained decreased 16:1ω9, 30% less EPA content, 18:2w6c, 14:0, and 18:3w3, but greater 16:0, 18:0, 5.5-fold greater DHA, ARA and 17:0 (SIMPER NW-WS: 79%). Chironomidae profiles in the two wood locations (WS and WW) also differed from one another, with WS having decreased EPA, 18:2ω6c and 14:0 but greater 16:1ω9, DHA, and ARA (SIMPER WS-WW: 57%).

A small number of taxa, primarily from the NW location, were located near the cluster of sediment and allochthonous resource profiles. The Plecoptera, Nemouridae, a shredder/gatherer stonefly found only in NW, had a profile highly similar to sediment (Fig. 5). While Ephemeroptera were found in all three mesohabitat locations, in WW *Baetis* diets were similar to sediment and TOM, and in NW *Caenis* and *Ephemera danica* had a profile similar to wood and leaves. The predatory

Coleopetera in NW, *Orectochilus villosus*, had a similar profile with the basal resources of sediment and wood and leaves, and probably fed on the nearby Plecoptera and Ephemeroptera in NW. The Heteroptera in NW, *Aphelocheirus aestivalis* larvae, while having a slightly different profile than most terrestrial sources, was most likely feeding on the Ephemeroptera present in NW. *Ephemera* found in WS was located away from other resources, in a cluster to the bottom right of the ordination. Several other taxa were located in this area, including NW Diptera (Chironomidae), and the predatory Heteroptera *A. aestivalis* in WS and the Odonata in both WS and NW locations which were probably feeding on these consumers in their respective locations.

Unionoida (*Unio* and *Anodonta*) and *Dreissena polymorpha* contained high levels of long-chain and branched fatty acids (*e.g.* 24:0, 22:2) and ARA, and with the exception of *D. polymorpha* in WS, grouped separately from all measured food resources. Despite feeding on seston, Unionoida and *D. polymorpha* profiles did not accurately represent the range of basal resources, as they are known to retain and possibly elongate commonly present fatty acids into long-chain branched forms which are not present or rare in seston (Gladyshev et al., 2011) and preferentially retain ARA (Newton *et al.*, 2013). However, these two mussel taxa had substantially different ω3:ω6 ratios, with *D. polymorpha* containing high ω3:ω6 (2.1±0.5) and Unio with low ω3:ω6 (0.7±0.1; Table S1).

The leech *Glossiphonia* sp., which was collected in only one sample, had a distinct fatty acid profile with extremely high levels of ARA (~26% of total FA; Table S1), most likely due to its particular feeding mechanism of sucking body fluids, and was very different from all food resources (outside plot viewing area).

## Discussion

The objectives of this study were to provide a methodological comparison of stable isotope and fatty acid food web methods, and to determine how the presence of large wood altered the trophic base of the macroinvertebrate consumer assemblage. Overall, the combination of stable isotope analysis with fatty acid biomarkers provided complementary data about how wood caused changes to the trophic base of the macroinvertebrate assemblage, and was particularly useful in addressing

the complex mix of lacustrine and riverine basal resources of both autochthonous and allochthonous origins.

Stable isotope data suggested that the biomass of the consumer assemblage in the studied reach of the Płociczna River was largely supported by the combined “seston and filamentous algae” resource across all wood-rich and wood-poor habitat locations. Fatty acid profiles, however, suggested filamentous algae were not a substantial part of the trophic base. Filamentous algae were only found in the wood-rich reach and were relatively rare, being restricted to a few small patches in over 300 m of channel. Therefore, even though seston and filamentous algae were combined isotopically within the stable isotope mixing model, this suggests that the dominant basal support was seston, which is likely to have originated from Lake Sitno located 700-1000m upstream of the sampling locations. The presence of wood clearly decreased the reliance of the macroinvertebrate assemblage on seston, as the trophic base shifted to use more epixylic autochthonous production by periphyton and bryophytes. Despite wood creating accumulations of detritus and organic matter in nearby sediments, stable isotopes suggest that this was relatively unimportant to the overall trophic base of the macroinvertebrate assemblage.

The results of the fatty acid biomarker profiles, in contrast, did not explicitly show a dominant seston signature supporting the macroinvertebrate assemblage. Instead, fatty acid profiles effectively discriminated between an autochthonous and allochthonous trophic base, primarily through HUFA content and ω3:ω6 ratios, and indicated that most consumer diets were supported by high-quality autochthonous production, such as bryophytes and periphyton. In addition to most consumer profiles being similar to these sources, most consumers maintained a ω3:ω6 ratio greater than 1, an indicator of a diet dominated by autochthonous matter (Torres-Ruiz, Wehr & Perrone, 2007;  Taipale, Kainz & Brett, 2015). Few consumers had profiles similar to terrestrial matter and sediment detritus, further reinforcing conclusions from the stable isotope data that allochthonous matter was a relatively unimportant part of the diet.

Fatty acid profiles suggesting a largely autochthonous trophic base do not explicitly contradict stable isotope data that suggest seston was a dominant part of the diet of many consumers.

Seston is a mix of allochthonous and autochthonous sources, and includes phytoplankton, bacteria, and processed terrestrial matter that may be present in various size fractions, and while fatty acid profiles may lack the resolution to distinguish between riverine or lacustrine origins (*e.g.* a "diatom signature" is similar whether from periphyton or phytoplankton sources: Dethier et al., 2013; Taipale et al., 2013), they can effectively descriminate between the origin of food resources (*i.e.* allochthonous or autochthonous). As a result, consumers that had stable isotope signatures indicating a predominantly seston diet and either an allochthonous or autochthonous fatty acid profile suggests selective feeding on particular fractions of the seston, a feeding behaviour noted in other studies (Thorp & Delong, 2002; Delong & Thorp, 2006). This is likely to be the case for the net-spinning caddisfly *Hydropsyche pellucidula*, which was estimated to have a similar diet dominated by seston (>75%) across all three habitat locations by stable isotope analysis, but fatty acid profiles detected diet differences between wood-rich and wood-poor habitats. Specifically, individuals collected from wood-rich substrata had greater ω3:ω6 ratios and higher tissue concentrations of HUFA and EPA, indicative of increased autochthonous matter in a seston-dominated diet. Wood provides an elevated position in the water column for *Hydropsyche* to attach their nets, and this may provide access to a more nutritive fraction of the seston than at the river-bed, as vertical stratification of transported particles has been suggested in low-slope, sand-bed rivers (Wright & Parker, 2004). This diet change may have implications for the success of *Hydropsyche* populations, as increased HUFA, and especially EPA, have been associated with increased *Hydropsyche* growth rates (Torres-Ruiz, Wehr & Perrone, 2010), and possibly other fitness measures such as survival and fecundity, as seen in other taxa (Müller-Navarra *et al.*, 2000; Kim, Arts & Yan, 2014; Taipale *et al.*, 2014).

Non-filter feeder taxa, such as *Baetis* sp. (mostly grazer and gatherer-collector), *Caenis* sp. (mostly gatherer-collector), Chironomidae (mostly gatherer-collectors, but with genus- or species-specific differences in feeding behaviours) and, to a lesser extent, Oligochaeta (mostly gatherer-collector), also showed a strong sestonic isotopic signature. This signature was most likely due to feeding on settling seston, which is enhanced in the low-flow areas around LW (Smock, Metzler & Gladden, 1989; Ehrman & Lamberti, 1992; Daniels, 2006; Cordova *et al.*, 2008), and also by the benthic-

pelagic coupling provided by filter feeder taxa (Wotton *et al.*, 1998; Vaughn, Gido & Spooner, 2004; Howard & Cuffey, 2006). Fatty acid profiles also indicated a shift to a more autochthonous diet at wood-rich sites for Chironomidae and Ephemeroptera. While this may be due to an actual shift in the specific diets of these taxa (Chapman & Demory, 1963; Rosi-Marshall & Wallace, 2002), it may also be a result of compositional changes to the available food resources across the three substrata. For example, Chironomidae show large genus- or species-specific differences in feeding behaviours (Ehrman & Lamberti, 1992), and thus the changes in diet that we recorded might be a result of sub-family shifts in taxonomic composition and their associated feeding preferences.

Consumers in this study had low $\delta^{13}C$, more $^{13}C$-depleted than most available food resources, and thus the seston value derived from *Unio* mussels was needed to resolve the consumer stable isotope signals. The $\delta^{13}C$ of *Unio*-derived seston was substantially lower than that of the >125 µm TOM fraction, and TOM was estimated to be a minimal part of the diet in the stable isotope mixing model. Such differences may be due to the high seasonal variability of the isotopic signature of lacustrine seston, with bulk isotopic values generally more $^{13}C$-depleted in winter and more enriched in spring (Zohary *et al.*, 1994). Therefore, Unionid mussel tissues may have partially retained this previous isotopic signature (Atkinson *et al.*, 2014; Cabana & Rasmussen, 1996) that was no longer present in the 125 µm TOM fraction at the time of sampling (April). Alternatively, the difference in the isotopic values may be due to a selective feeding behaviour of the unionid mussels on different fractions of the seston (*e.g.* ultra-fine nutritive particles, such as $^{13}C$-depleted bacteria) or to the pedal feeding behaviour of mussels (Nichols & Garling, 2000). Previous studies have suggested that seston <100 µm in size is in fact more $^{13}C$-depleted than seston >100 µm (Delong & Thorp, 2006), which further supports the idea of size-selective feeding by both unionid mussels and other consumers with similarly low stable isotope values. However, since other studies suggest that unionid mussels can feed on particles of a broad size range up to 250 µm (Vaughn, Gido & Spooner, 2004), further investigation is required.

Differences in the fatty acid profiles between Unionid and *Dreissena* mussels, particularly in the ω3:ω6 ratio, may suggest taxa-specific feeding ecologies on different fractions of the seston. However, fatty acids may be ineffective at directly determining mussel diets due to the noted ability for mussels to preferentially retain or modify fatty acids into forms which are not present or rare in the seston (Gladyshev *et al.*, 2011), including ARA (Newton *et al.*, 2013). While *Dreissena* fatty acid profiles have been shown to reflect changes in catchment land-use (Larson *et al.*, 2013) and habitats in large rivers (Larson *et al.*, 2015), without further research into the process of fatty acid trophic modification by mussels, fatty acid profiles may be less applicable for directly determining the specific composition of mussel diets than for other consumers.

The strong influence of lacustrine seston from Lake Sitno on the trophic base of benthic macroinvertebrates in the Płociczna river effectively subsidised an increase in biomass in downstream assemblages (Richardson & Mackay, 1991; Hillbricht-Ilkowska, 1999) and created a strong coupling between lake and river productivity (Perry & Sheldon, 1986; Junger & Planas, 1994). The lower $\delta^{13}C$ of seston and high levels of bacterial fatty acids in the food web suggests that this lacustrine carbon is likely produced via a microbial link, *i.e.* bacteria-flagellate-ciliate-*Daphnia*; (Kankaala *et al.*, 2006). This lake-derived carbon then enters the river ecosystem and provides a cross-ecosystem food subsidy for benthic consumers that may be otherwise limited by low local primary productivity (Perry & Sheldon, 1986; Junger & Planas, 1994). River-lake coupling is expected to be particularly strong in lowland sand-bed rivers and in other lowland rivers with fine, unstable sediments, as sediment instability limits in-stream primary production to support overall ecosystem productivity (Atkinson *et al.*, 2008).

However, a recent study has shown that production based on a predominantly bacterial carbon source is highly limited by the availability of physiologically essential lipids and fatty acids derived from algal production, without which bacteria cannot support zooplankton productivity (Taipale *et al.*, 2014). Therefore, an increase in carbon subsidies from either bacterial or terrestrial sources without addressing a limiting availability of physiological essential fatty acids may result in little change to secondary production. Indeed, large wood created accumulations of organic matter and

allochthonous resources (*i.e.* leaves) which were otherwise minimal and limited to marginal areas of the channel, yet this oft-noted ability for wood to accumulate organic matter (Smock, Metzler & Gladden, 1989) had little effect on the trophic base of the macroinvertebrate assemblage, most likely due to its low-nutritional quality. In contrast, large wood dramatically increased the amount of stable substratum for colonisation by periphyton and bryophytes (Golladay & Sinsabaugh, 1991; Hax & Golladay, 1993; Wondzell & Bisson, 2003), and thus acted as a hotspot of otherwise limited high-nutritional quality autochthonous production. In addition, large wood also served as an attachment site for the net-spinning caddisfly *Hydropsyche* to feed on a more nutritive and autochthonous component of the seston. Overall, wood increased the contributions of high-quality autochthonous primary production to the trophic base of the macroinvertebrate assemblage.

These results are in contrast to a recent study using gut-content analysis which suggested that the trophic base around wood was mainly supported by transported amorphous detritus, presumably of allochthonous origin (Benke & Bruce Wallace, 2015). While we also found that transported material provided a large contribution to the diet, our fatty acid data suggest that the seston and drifting detritus consumed were primarily of autochthonous, and not allochthonous, origin. Overall, the combined stable isotope and fatty acid approach contained in this study supports previous work emphasising the importance of high quality autochthonous resources for riverine productivity (Thorp & Delong, 2002), even in light-limited rivers that contain high terrestrial inputs (Torres-Ruiz, Wehr & Perrone, 2007; Lau, Leung & Dudgeon, 2008; Lau, Leung & Dudgeon, 2009).

In conclusion, our study showed that SIA and FA analyses complement each other and thus their combined use can improve studies of freshwater food webs, particularly where resources may be a complex mix of lacustrine and riverine origins. Since stable isotopes are subjected to seasonal and local variation, SIA can identify the contribution of food resources of different spatial origin (*i.e.* riparian zone, lake and river), but may be difficult to interpret due to seasonal changes and be unable to separate resources with similar isotopic signatures. On the other hand, fatty acids can be used to accurately estimate taxonomic groupings even with seasonal variations (Dethier *et al.*, 2013; Taipale *et al.*, 2013), although they do not distinguish between lacustrine or riverine origins.

484 The results presented in this paper demonstrate that the presence of large wood decreases the
485 strength of river-lake coupling by providing alternative basal resources, primarily through its role as
486 a hard substratum supporting colonisation by periphyton/bryophytes and hence increasing local,
487 high-quality autochthonous productivity. As the influence of lake subsidies decreases at increasing
488 distance from the lake (Richardson & Mackay, 1991; Hillbricht-Ilkowska, 1999), the role of large
489 wood is likely to increase, as secondary production would be entirely dependent on the remainder
490 of locally-produced food resources. Thus, river management that would affect the availability of
491 wood and its effects on habitat heterogeneity would ultimately alter patterns of energy flow and
492 ecosystem productivity by changing the availability and nutritional-quality of basal food resources.

493 **Acknowledgments**

494 We thank Jürgen Schreiber for assistance in the field, Angela Kruger and Katrin Premke for
495 assistance in the laboratory, Angela Gurnell for support with travel and logistics, and Mario Brauns
496 for help with the stable isotope mixing models. This research was carried out within the Erasmus
497 Mundus Doctorate Program SMART (http://www.riverscience.eu) funded by the Education,
498 Audiovisual and Culture Executive Agency (EACEA) of the European commission.

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## Tables

**Table 1:** Taxa collected in the three mesohabitat locations in the Płociczna River, along with the dominant functional feeding group and the assimilation analysis test conducted. NW= river-bed sediments in the wood-poor site; WS= river-bed sediments in the wood-rich site; WW= the wood surface in the wood-rich site. Shr=shredders; Grz=grazers; Prd=predator; Gat=gatherers; AFF=active filterers; PFF=passive filterers; Min=miners.

| Taxon | Functional Group | Analysed for SIA | Analysed for FA | NW | WS | WW |
|---|---|---|---|---|---|---|
| *Anabolia* sp. | Shr/Grz/Prd/Gat | X | | X | | |
| *Anodonta anatina* | AFF | X | X | X | | |
| *Aphelocheirus aestivalis* adult | Pred | X | X | | X | |
| *Aphelocheirus aestivalis* larvae | Pred | X | X | X | X | X |
| *Asellus aquaticus* | Gat/Grz/Shr | X | | X | | |
| *Baetis* sp. | Grz/Gat | X | X | | X | X |
| *Bithynia tentaculata* | AFF/Grz/Gat | X | X | X | X | |
| *Caenis* sp. | Gat | X | X | X | X | X |
| *Calopteryx* sp. | Prd | X | | X | x | |
| Chironomidae | Gat/AFF/Grz/Min/Prd | X | X | X | X | X |
| *Dreissena polymorpha* | AFF | X | X | X | X | |
| *Ephemera danica* | AFF/Gat | X | X | X | X | X |
| *Gammarus pulex* | Shr/Gat/Grz/Prd | | X | | X | |
| *Gammarus roselii* | Shr/Gat/Grz/Prd | | X | | X | |
| *Glossiphonia* sp. | Prd | X | X | X | | |
| *Gomphus* sp. | Prd | X | X | X | X | |
| *Hydropsyche pellucidula* | PFF/Prd/Grz | X | X | X | X | X |
| Limnephilidae | Shr/Grz/Prd/Gat | X | | | X | |
| Nemouridae | Shr/Gat | X | X | X | | |
| *Neureclipsis bimaculata* | PFF/Prd | X | | | X | |
| Oligochaeta | Gat | X | | X | X | |
| *Ophigomphus cecilia* | Prd | X | X | X | | |
| *Orectochilus villosus* | Prd | X | X | X | X | X |
| *Platycnemis* sp. | Prd | X | X | X | | |
| *Polycentropodidae* | Prd / PFF | X | X | X | X | X |
| *Potamopyrgus antipodarum* | Oth/Gat/Shr/Graz | X | X | X | | |
| *Psychomia pusilla* | Grz/Gat/PFF/Prd | X | | | | X |
| Sphaeriidae | AFF | X | X | X | X | X |
| Tabanidae | Prd | X | X | X | X | |
| *Theodoxus fluviatilis* | Grz | X | | X | | |
| *Unio pictorum* | AFF | X | X | X | X | |
| *Unio tumidus* | AFF | X | X | X | X | |

712 **Table 2:** Fatty acid composition data for the various food resources. Values indicate mean and standard error. Total fatty acid content is given by
713 weight (mg $g^{-1}$) and indicates the sum of all quantified FA, and the subdivision into the 4 main fatty acid classes is indicated by percentage of all
714 quantified fatty acids. No significant differences were seen between wood-rich and wood-poor locations, and data is the average across locations.
715 SAFA = saturated fatty acids. MUFA = monounsaturated fatty acids. PUFA = polyunsaturated fatty acids. HUFA = highly unsaturated fatty acids.
716 EPA = eicosapentaenoic acid; 20:5ω3, DHA = docosahexaenoic acid; 22:6ω3, and ARA = arachidonic acid; 20:4ω6. BrFA = the sum of quantified
717 bacterial fatty acids (15:0, 17:0). ω3:ω6 = the ratio of the sum of all omega-3 to omega-6 fatty acids. Letters indicate post-hoc significant
718 differences at $P < 0.05$.

| Basal Resources | Total FA Content | Proportion of Fatty Acid Classes (%) | | | | Specific Fatty Acid Biomarkers (%) | | | | Biomarker Ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| | (mg $g^{-1}$) | SAFA | MUFA | PUFA | HUFA | EPA | DHA | ARA | BrFA | ω3:ω6 |
| Mussel periphyton | 51.93±4.97$^{a}$ | 55.1±1.5$^{a}$ | 14.3±2.5$^{d}$ | 11.5±0.7$^{ab}$ | 19.1±0.9$^{a}$ | 12.4±0.8$^{a}$ | 2.3±0.1$^{ab}$ | 1.7±0.1$^{ab}$ | 2.2±0.3$^{b}$ | 3.5±0.1$^{a}$ |
| Wood periphyton | 44.32±22.82$^{ab}$ | 60.7±1.0$^{a}$ | 17.9±0.6$^{cd}$ | 8.4±0.4$^{abc}$ | 13.1±0.6$^{ab}$ | 8.0±0.8$^{ab}$ | 1.6±0.4$^{abc}$ | 1.6±0.2$^{ab}$ | 4.4±0.9$^{ab}$ | 2.5±0.3$^{ab}$ |
| Bryophyte | 40.87±8.76$^{ab}$ | 56.6±5.6$^{a}$ | 18.3±2.9$^{cd}$ | 10.9±0.8$^{abc}$ | 14.3±1.5$^{a}$ | 7.6±1.3$^{ab}$ | 2.5±0.7$^{a}$ | 2.7±0.3$^{a}$ | 3.4±0.5$^{ab}$ | 1.9±0.3$^{ab}$ |
| Grass | 16.82±3.24$^{bc}$ | 58.7±1.7$^{a}$ | 22.4±1.9$^{abc}$ | 14.3±2.3$^{a}$ | 4.6±0.6$^{c}$ | 2.5±0.4$^{c}$ | 0.4±0.1$^{cd}$ | 0.9±0.1$^{b}$ | 4.4±0.7$^{ab}$ | 0.8±0.1$^{c}$ |
| Wood & leaves | 15.66±2.35$^{c}$ | 59.3±1.5$^{a}$ | 21.4±0.7$^{bc}$ | 11.3±1.1$^{abc}$ | 8.0±1.1$^{bc}$ | 4.6±0.8$^{bc}$ | 0.7±0.1$^{bcd}$ | 1.4±0.2$^{b}$ | 3.9±0.4$^{ab}$ | 1.0±0.1$^{c}$ |
| TOM | 10.20±2.44$^{c}$ | 63.3±1.6$^{a}$ | 22.1±0.4$^{abc}$ | 7.2±0.5$^{bc}$ | 7.5±1.5$^{bc}$ | 3.8±0.7$^{bc}$ | 1.2±0.4$^{abc}$ | 1.3±0.2$^{b}$ | 5.3±0.6$^{a}$ | 1.4±0.2$^{bc}$ |
| Filamentous algae | 9.85±4.23$^{c}$ | 52.6±1.3$^{a}$ | 32.7±2.7$^{a}$ | 5.7±1.1$^{c}$ | 9.1±0.6$^{abc}$ | 6.1±0.2$^{abc}$ | 0.3±0.2$^{d}$ | 1.8±0.3$^{ab}$ | 4.3±1.5$^{ab}$ | 1.6±1.0$^{abc}$ |
| Sediment | 5.68±2.43$^{c}$ | 59.6±1.2$^{a}$ | 25.5±0.7$^{ab}$ | 7.3±0.7$^{bc}$ | 7.7±0.9$^{bc}$ | 4.3±0.6$^{bc}$ | 0.6±0.1$^{cd}$ | 1.6±0.2$^{ab}$ | 4.2±0.3$^{ab}$ | 1.2±0.2$^{bc}$ |

719 **Figure legends**

720 Figure 1: Map of the study area. The Płociczna flows through a series of lakes before its
721 confluence with the Drawa, and both wood-poor and wood-rich sampling locations were located
722 700 m – 1 km downriver of the outlet of Lake Sitno.

723 Figure 2: Non-metric multidimensional scaling of the macroinvertebrate assemblage composition in
724 the three sampled substrates (WW: wood surface in the wood-rich site; WS: river-bed sediment
725 surrounding wood in the wood-rich site; NW: river-bed sediment in the wood-poor site), performed
726 on log(x+1)-transformed abundances and with Bray-Curtis distance.

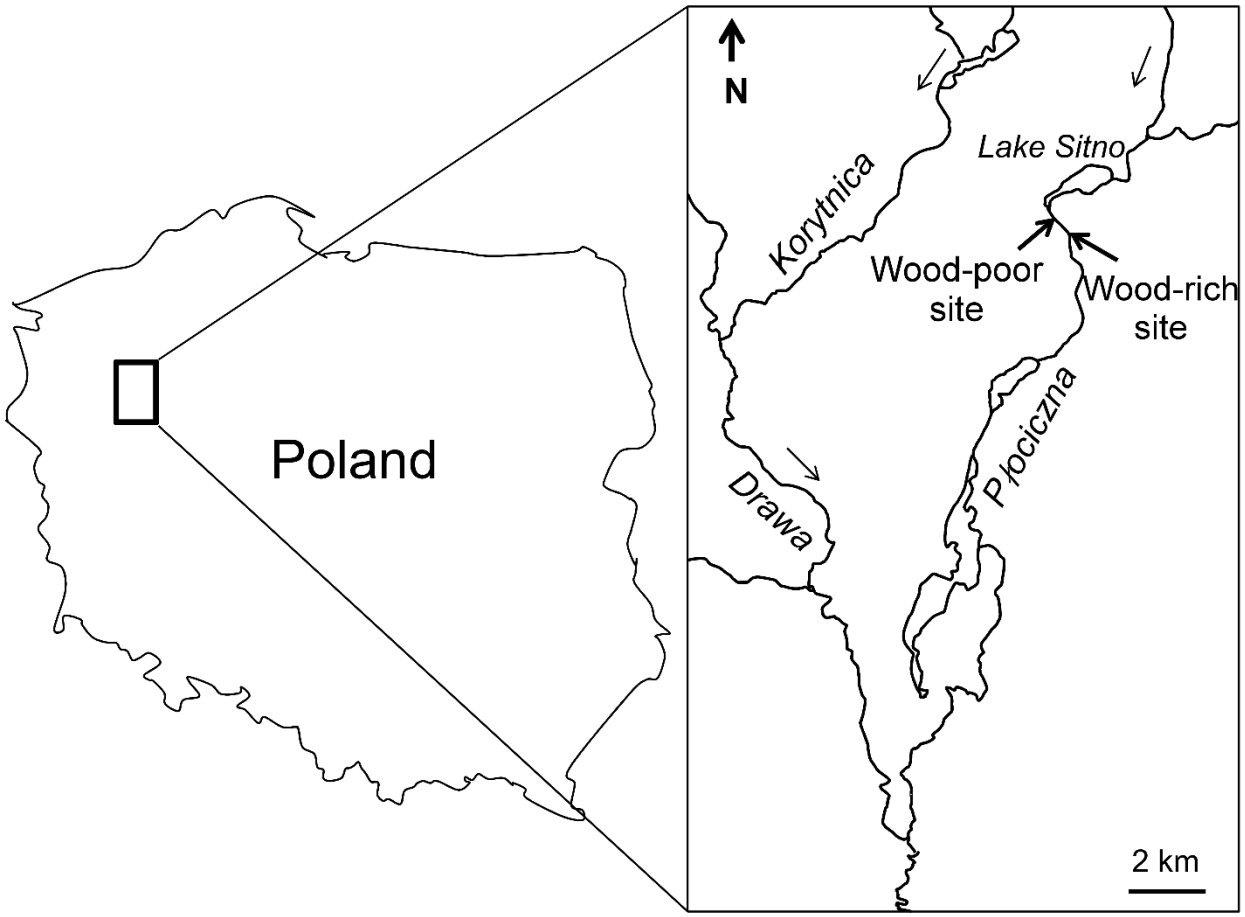
727 Figure 3: Stable carbon and nitrogen isotope signatures of resources (lines, mean ± s.d.) and
728 macroinvertebrates (circles) in the wood-poor site (NW, dotted lines and open circles) and in the
729 wood-rich site (W, solid lines and solid circles). The isotopic signatures of macroinvertebrates were
730 corrected by trophic enrichment factors of 0.4 ± 1.3 ‰ for $\delta^{13}C$ and 3.4 ± 1.0 ‰ for $\delta^{15}N$ (Post,
731 2002); those values were doubled for predator taxa. Resources abbreviations: FilA= filamentous
732 algae; Bry= bryophytes; TOM= transported organic matter; SM= seston inferred from the isotopic
733 signature of unionid mussels (see text for explanation); PeriW= periphyton on wood;
734 PeriM= periphyton on the shells of unionid mussels; D= detritus; W= wood; G= grass; L= leaves.

735 Figure 4: Contributions of the trophic resources to the total biomass of the macroinvertebrate
736 assemblage on the three substrates (WW: wood surface in the wood-rich site; WS: river-bed
737 sediment surrounding wood in the wood-rich site; NW: river-bed sediment in the wood-poor site).
738 Mean values ± 95% credible interval. Only the three groups of food resources which contributed
739 the most to macroinvertebrate biomass are shown: SesM-FilA= seston inferred from Unionids (see
740 text for explanation) and filamentous algae, PeriW-Bry= periphyton on wood and bryophytes, Gr-L=
741 grass and leaves.

742 Figure 5: Non-metric dimensional scaling of all basal resources and collected macroinvertebrates
743 in the study locations. Ellipses represent 95% confidence intervals around the centroid for each
744 basal resource. Ordispider lines are used for filamentous algae (Fil A) and transported organic
745 matter (TOM) to indicate high variability in the samples. Consumer values (symbol) represent the
746 centroid and 1 standard error, and consumer proximity with food resources indicate feeding
747 preferences. All consumers are grouped by order in the figure to aid interpretation of the figure,
748 except for Sphaeriidae and *D. polymorpha*. Glossiphoniidae is outside of viewable chart area. WW
749 (wood surface in the wood-rich site; black fill), WS (river-bed sediments surrounding the wood;
750 grey fill), and NW (river- bed sediment in the wood-poor site; white fill). Bry = bryophytes. PeriM =
751 periphyton on the shells of unionid mussels. PeriW = periphyton on the surface of wood. Gr =
752 Grass. W&L = wood and leaves. Sed = Sediment

753

754 **Figures**

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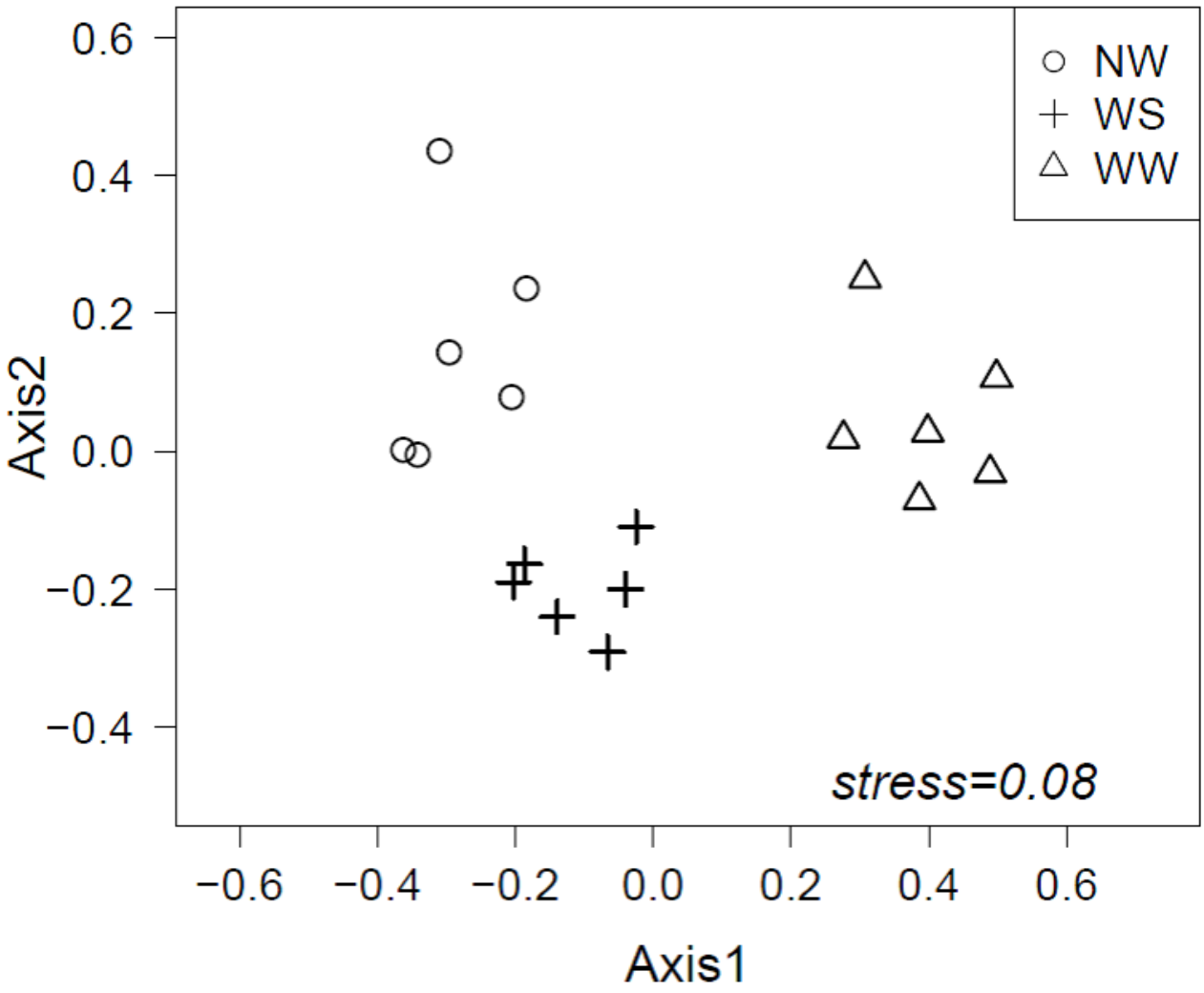
756 Figure 1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WS
WW
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Axis2
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0.2
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−0.6
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−0.2
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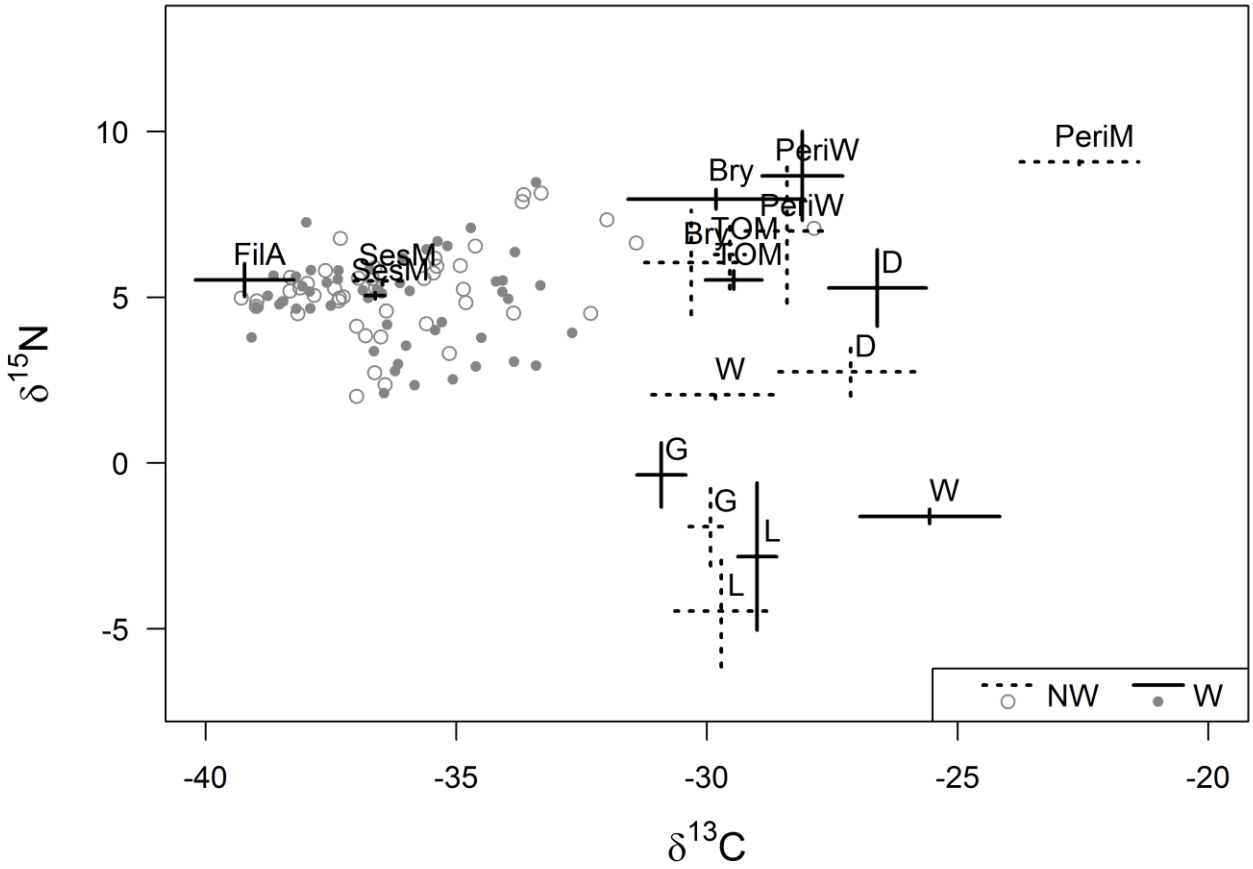
0.2
0.4
0.6

Figure 2




760
761 Figure 3

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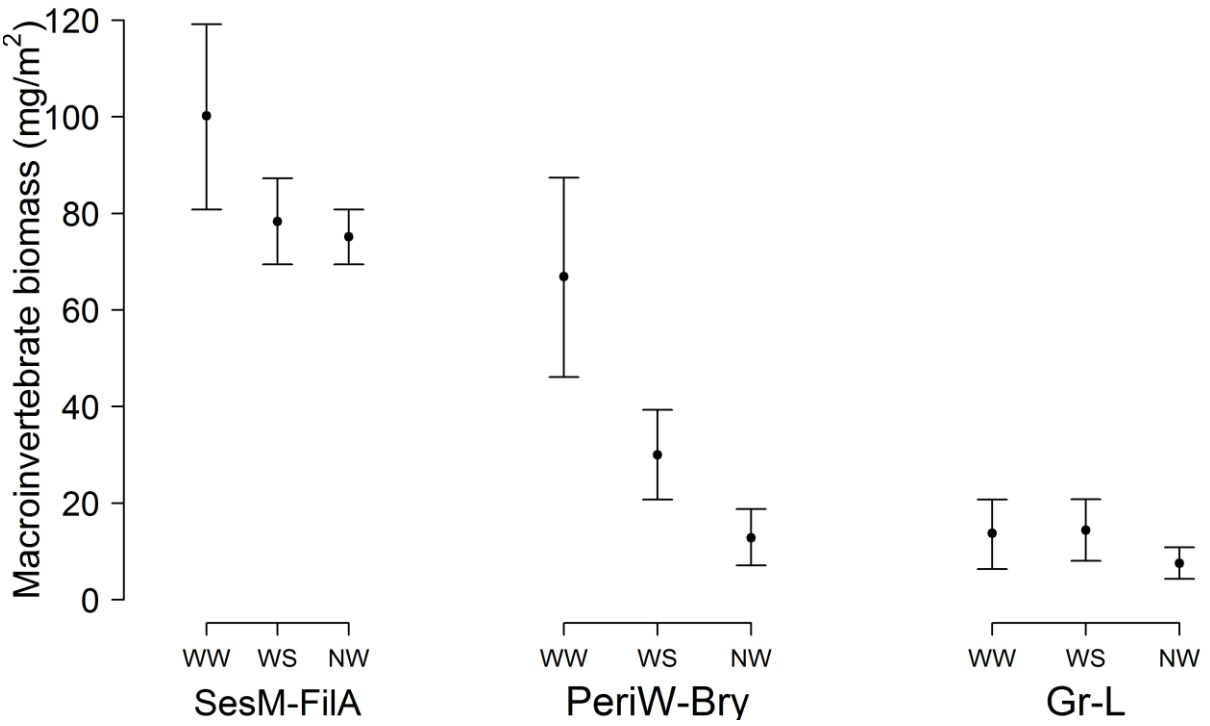
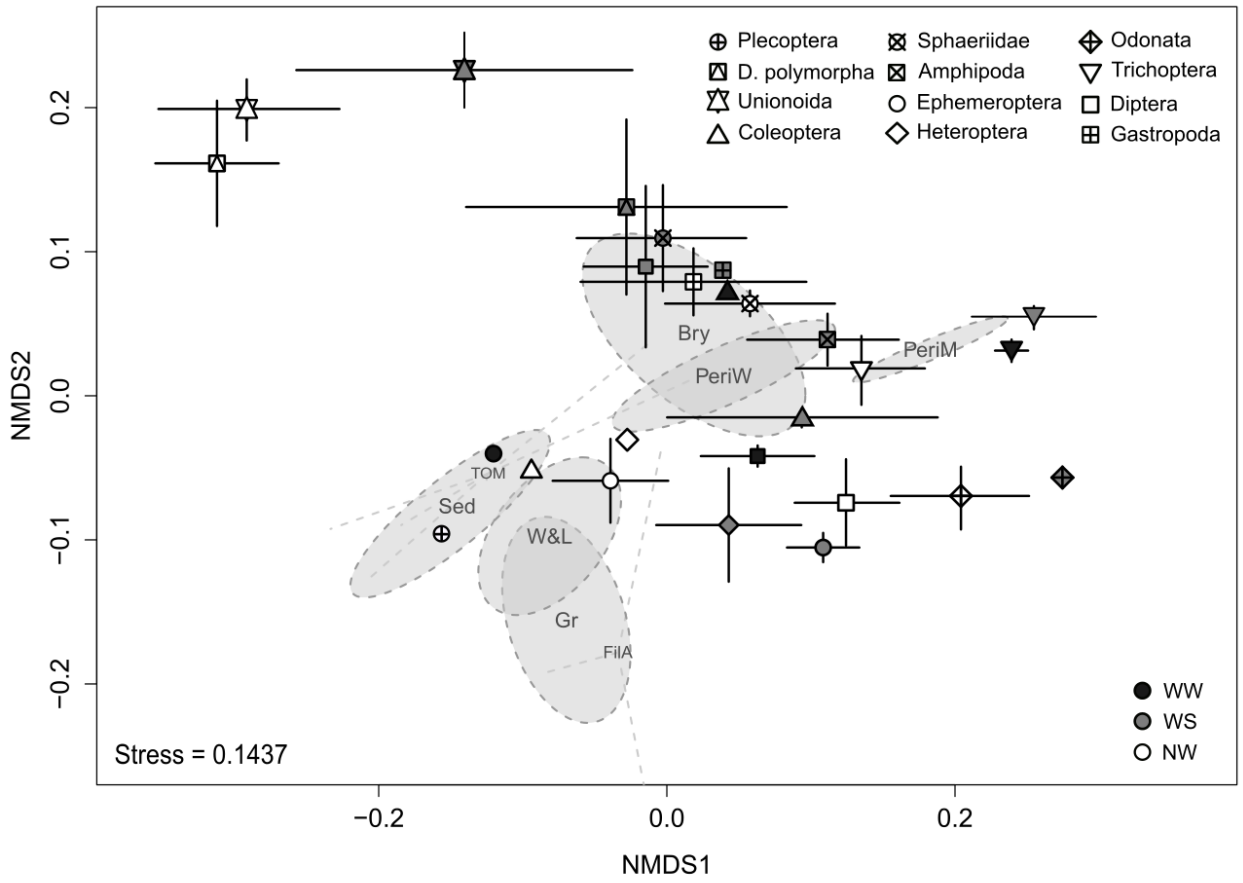



763

764 Figure 4

765




766
767 Figure 5

768

769 **Appendices (Supporting Information)**
770

771

772 **Figure S1:** Pictures taken in both the wood-rich (top) and wood-poor (bottom) sites in the
773 Płociczna River looking downriver from mid-channel. Each site was a 100 m long reach, and 300
774 m separated the two sites. Macroinvertebrates were sampled in the wood-rich site on the wood
775 surface (WW) and in sediment surrounding wood (WS), and in the wood-poor site in river-bed
776 sediment (NW).

777

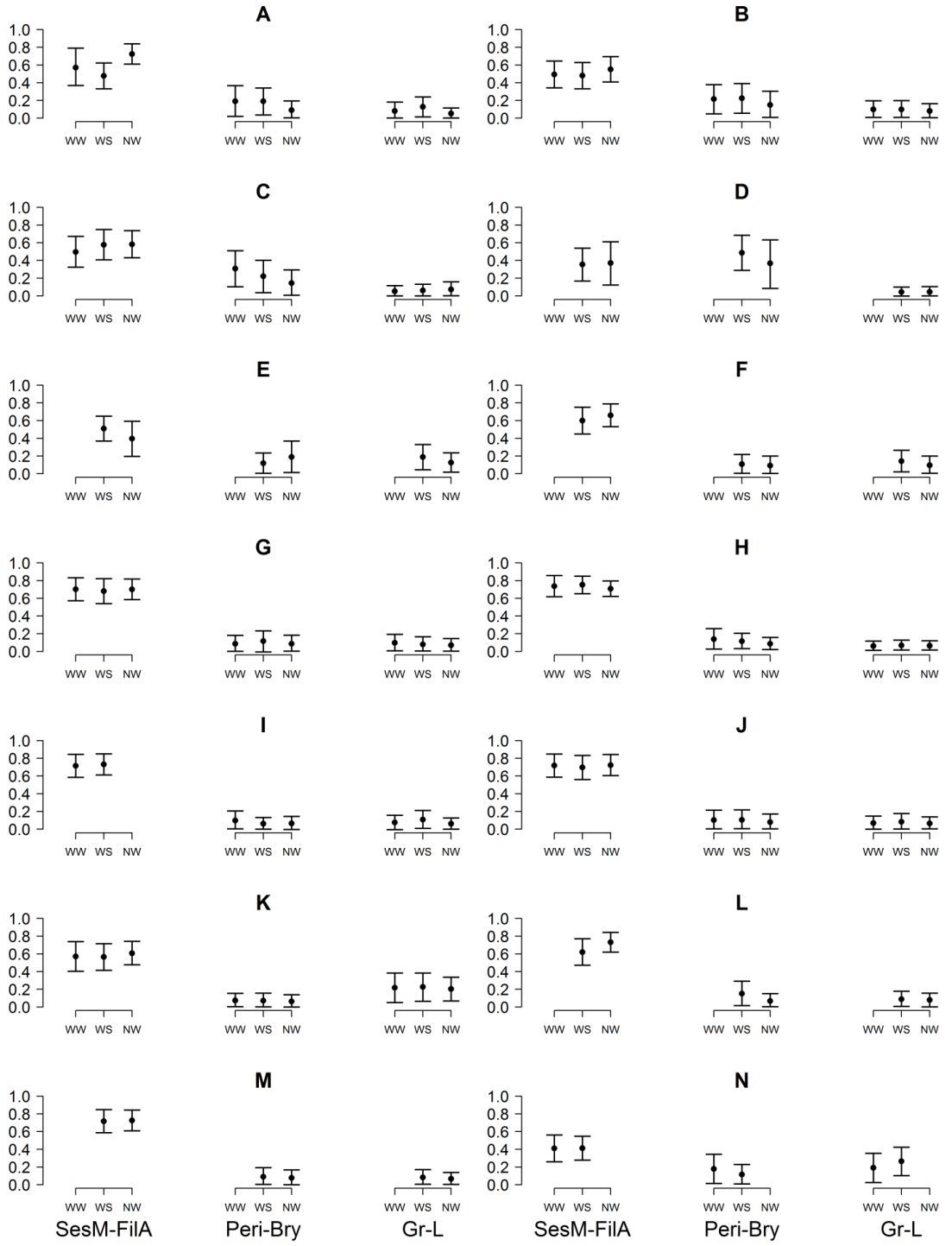



**Figure S2:** Relative contributions of the basal trophic resources to the diet of collected macroinvertebrates in the three sampling substrates (WW: wood surface; WS: river-bed sediments surrounding the wood; NW: river-bed sediment in the wood-poor site) according to the result of the isotopic mixing model (see text for details). Mean values ± 95% credible interval. Figure only shows results obtained for taxa collected in both wood and non-wood sites, and only displays trophic resources which contributed >10% to the diet of at least one taxon. SesM-FilA= seston inferred from Unionids (see text for explanation) and filamentous algae, PeriW-Bry= periphyton on wood and bryophytes, Gr-L= grass and leaves. A: Chironomidae, B: *Caenis* sp., C: *Ephemera danica*, D: Oligochaeta, E: *Bithynia tentaculata*, F: Gomphidae, G: Sphaeriidae, H: *Hydropsychae* sp., I: *Orectochilus villosus*, L: Policentropodidae, M: *Aphelocheirus aestivalis*, N: Tabanidae, O: *Dreissena polymorpha*, P: Baetis sp.

**Table S1:** Fatty acid composition data for all consumers. Values indicate percentage mean and standard error. SAFA = saturated fatty acids. MUFA = monounsaturated fatty acids. PUFA = polyunsaturated fatty acids. HUFA = highly unsaturated fatty acids. EPA = eicosapentaenoic acid; 20:5ω3, DHA = docosahexaenoic acid; 22:6ω3, and ARA = arachidonic acid; 20:4ω6. BrFA = the sum of quantified bacterial fatty acids (*i.e.* 15:0, 17:0). ω3:ω6 = the ratio of the sum of all omega-3 to omega-6 fatty acids. Letters indicate post-hoc significant differences between sampling substrates at $P < 0.05$.

| Group | | N | SAFA% | MUFA% | PUFA% | HUFA% | EPA% | DHA% | ARA% | ω3:ω6 | BrFA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A. aestivalis adult | | 2 | 43.5±4.1 | 21.1 ±4.4 | 15.2 ±0.1 | 20.3 ±0.5 | 13.1 ±0.4 | 0.9 ±0.5 | 4.2 ±0.4 | 1.4 ±0.1 | 2.3±0.2 |
| A. aestivalis larvae | | 3 | 61.3 ±6.7 | 18.3 ±4.6 | 9.3 ±1.1 | 11.0 ±1.0 | 7.3 ±0.6 | 0.3 ±0.2 | 2.0 ±0.2 | 1.4 ±0.1 | 2.9±0.7 |
| Baetis | | 2 | 60.3 ±1.2 | 21.9 ±2.5 | 8.0 ±0.9 | 9.7 ±0.3 | 7.5 ±0.3 | 0.2 ±0.1 | 1.0 ±0.2 | 2.4 ±0.4 | 4.1±0.0 |
| Bithynia | | 3 | 53.4 ±0.4 | 14.0 ±2.2 | 9.8 ±0.8 | 22.8 ±1.7 | 9.8 ±1.6 | 5.3 ±1.0 | 4.8 ±0.5 | 2.2 ±0.4 | 3.4±0.5 |
| Caenis | | 3 | 51.0 ±4.7 | 22.8 ±0.6 | 9.9±1.1 | 16.3 ±4.1 | 10.3 ±2.4 | 0.4 ±0.1 | 3.9 ±1.6 | 1.3 ±0.1 | 3.8±0.1 |
| Chironomidae | NW | 3 | 46.4 ±2.5 | 23.8 ±0.2 | 14.8 ±1.4 | 15.0 ±1.0 | 11.4 ±0.8$^{a}$ | 0.6 ±0.2 | 1.7 ±0.3 | 1.7 ±0.2 | 1.4±0.5$^{a}$ |
| | WS | 3 | 55.0 ±0.1 | 18.0 ±2.1 | 10.3 ±1.3 | 16.7 ±3.0 | 6.6 ±0.7$^{b}$ | 3.9 ±2.1 | 4.1 ±1.3 | 1.5 ±0.4 | 5.0±0.7$^{b}$ |
| | WW | 3 | 52.7 ±2.7 | 18.5 ±3.1 | 13.0 ±0.1 | 15.9 ±1.7 | 11.8 ±1.4$^{a}$ | 0.9 ±0.2 | 1.7 ±0.1 | 1.7 ±0.2 | 2.8±0.3$^{ab}$ |
| Dreissena | | 5 | 60.2±11.0 | 23.2 ±11.1 | 4.6 ±2.0 | 11.8 ±2.5 | 3.6 ±1.8 | 3.1 ±0.9 | 1.5 ±0.5 | 2.1 ±0.5 | 3.2±0.5 |
| Ephemera | | 5 | 51.5 ±2.6 | 20.5 ±1.9 | 10.0 ±0.9 | 18.1 ±2.1 | 12.4 ±1.9 | 0.9 ±0.3 | 3.2 ±0.2 | 2.0 ±0.2 | 3.4±0.4 |
| Gammarus | | 2 | 51.4 ±1.8 | 15.9 ±2.2 | 11.8 ±0.7 | 20.9 ±0.3 | 12.5 ±1.0 | 3.0 ±0.8 | 2.7 ±0.8 | 2.6 ±0.5 | 3.2±0.7 |
| Glossiphonia | | 1 | 22.1 | 22.4 | 6.2 | 49.3 | 10.9 | 2.9 | 26.2 | 0.6 | 2.0 |
| Gomphidae | | 4 | 51.2 ±0.9 | 10.2 ±2.4 | 16.8 ±1.5 | 21.8 ±0.7 | 16.3 ±0.9 | 1.3 ±0.2 | 2.9 ±0.1 | 2.6 ±0.1 | 1.5±0.2 |
| Hydropsyche | NW | 3 | 57.7 ±1.8$^{a}$ | 17.7 ±2.0 | 10.2 ±0.4$^{a}$ | 14.4 ±0.6$^{a}$ | 9.7 ±0.2$^{a}$ | 1.4 ±0.3$^{a}$ | 1.5 ±0.1 | 2.9 ±0.1$^{a}$ | 4.2±1.0$^{a}$ |
| | WS | 6 | 50.8 ±1.7$^{b}$ | 14.6 ±1.2 | 12.4 ±0.7$^{b}$ | 22.3 ±1.1$^{b}$ | 13.8 ±0.6$^{b}$ | 3.2 ±0.2$^{b}$ | 1.8 ±0.1 | 3.8 ±0.1$^{b}$ | 3.0±0.6$^{a}$ |
| | WW | 7 | 50.2 ±1.1$^{b}$ | 15.4 ±1.2 | 12.6 ±0.3$^{b}$ | 21.8 ±0.8$^{b}$ | 14.6 ±0.6$^{b}$ | 2.4 ±0.2$^{c}$ | 1.7 ±0.1 | 3.8 ±0.1$^{b}$ | 2.6±0.4$^{a}$ |
| Nemouridae | | 1 | 59.1 | 25.7 | 8.6 | 6.6 | 3.5 | 0.5 | 1.5 | 0.8 | 5.7 |
| Orectochilus | | 4 | 50.4 ±3.9 | 22.4 ±2.4 | 12.6 ±1.2 | 14.6 ±2.8 | 9.8 ±2.1 | 1.0 ±0.3 | 1.2 ±0.2 | 2.8 ±0.4 | 2.8±0.3 |
| Platycnemis | | 1 | 55.5 | 14.9 | 12.6 | 17.0 | 12.4 | 0.4 | 3.3 | 1.6 | 3.2 |
| Polycentropodidae | | 2 | 48.1 ±1.1 | 15.2 ±7.4 | 13.1 ±0.3 | 23.6 ±6.1 | 14.3 ±2.4 | 4.2 ±3.0 | 1.8 ±0.2 | 3.7 ±1.2 | 2.0±0.1 |
| Potamopyrgus | | 1 | 59.7 | 19.8 | 7.3 | 13.3 | 5.0 | 2.2 | 3.7 | 1.3 | 3.9 |
| Sphaerium | | 5 | 52.6 ±2.9 | 19.4 ±1.5 | 10.5 ±1.3 | 17.5 ±2.1 | 6.6 ±1.4 | 4.5 ±0.6 | 3.9 ±0.6 | 1.7 ±0.2 | 4.0±0.6 |
| Tabanidae | | 1 | 59.8 | 21.5 | 7.6 | 11.2 | 5.8 | 1.9 | 1.9 | 1.9 | 4.8 |
| Unionidae | | 10 | 64.5 ±6.0 | 13.6 ±1.6 | 5.6 ±1.1 | 16.3 ±3.5 | 4.5 ±1.6 | 2.6 ±0.9 | 5.3 ±1.7 | 0.7 ±0.1 | 5.0±0.3 |